

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2211510/EJH/AF	<div style="display: flex; justify-content: space-between;"> <div style="text-align: center;">FOR FURTHER ACTION</div> <div>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</div> </div>	
International application No. PCT/AU 99/00931	International filing date (<i>day/month/year</i>) 27 October 1999	(Earliest) Priority Date (<i>day/month/year</i>) 27 October 1998
Applicant <div style="text-align: center; font-weight: bold;">THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH et al</div>		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **4** sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international application, the international search was carried out on the basis of the sequence listing:
- ☐ contained in the international application in written form.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority in written form.
 - ☐ furnished subsequently to this Authority in computer readable form.
 - ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title, ☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract, ☒ the text is approved as submitted by the applicant

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- ☐ as suggested by the applicant.
☐ because the applicant failed to suggest a figure
☐ because this figure better characterizes the invention

☐ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00931

A. CLASSIFICATION OF SUBJECT MATTERInt Cl⁶:

A61K 38/16, 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K WITH ELECTRONIC DATABASE AS INDICATED BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU : IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLU/ MEDLINE/ WPAT:

1) EPHRN OR SEK 1 OR EPHA4 2) 1 AND AXON 3) 1 AND NEUR 4) 1 AND NERV 5) 2 OR 3 OR 4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO-A-96/09384 (President & Fellows of Harvard College) 28 March 1996 - partic pages 47-51	1-10,12, 18, 20, 21 11,13,14-17, 19
X Y	WO-A- 96/23000 (Amgen Inc) 1 August 1996 - in partic page 16 claims 30, 31	1-10,12, 18, 20,21 11,13, 14-17, 19
X Y	WO 96/13518 (Genentech) 9 May 1996 - in partic pages 22-25, claims 24, 25	1-10, 12, 18, 20, 21 11,13,14-17, 19

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 November 1999

Date of mailing of the international search report

17 DEC 1999

Name and mailing address of the ISA/AU

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C (Classification). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 96/26958 (President & Fellows of Harvard College) 6 September 1996 -in part pages 25-26, claims 40-48, 58.	1-10,12,18,20,21 11,13,14-17,19
X Y	WO 97/04091 (Amrad Operations Pty Ltd) 6 February 1997 - in part page 24.	1-12, 18, 20,21 13, 14-17, 19
X Y	WO 97/14966 (Mt Sinai Hospital Corporation) 24 April 1997 - in part pages 3, 17, 18.	1-10, 12, 14-18, 20,21 11, 13,19
X Y	EMBO Journal, Vol 16 No11 (June 1997), pages 3106-3114, "Aberrant Axonal Projections in Mice Lacking EphA8 (Eek) Tyrosine Protein Kinases Receptors", Park et al	14-17 1-13, 18-21
Y	Curr. Biol, Vol 7 No 8 (August 1997). Pages 561-570, "The EphA4 and EphB1 Receptor tyrosine Kinases and Ephrin-B2 Ligand regulate targeted Migration of Branchial Neural Crest Cells", Smith et al	1-21
Y	Exercise and Sport Science Reviews, Vol 22 (1994) pages 361-388, "Transgenic Animal Models", Tsika.	1-21
X Y	Cur. Opin. Neuro., Vol 8 No 1 (February 1998) pages 117-127, "Cell-Contact-Dependent Signalling in Axon Growth and Guidance: Eph Receptor Tyrosine Kinases and Receptor Protein tyrosine phosphatase β ", Holland et al.	14-17 1-13, 18-21

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00931

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	9623000	AU	49047/96	CA	2210644		
WO	9704091	AU	64098/96	EP	842272		
WO	9613518	AU	44053/96	CA	2203888	EP	787148
WO	9609384	AU	35920/95	US	5795734		

END OF ANNEX



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 38/16, 38/00	A1	(11) International Publication Number: WO 00/24413 (43) International Publication Date: 4 May 2000 (04.05.00)
(21) International Application Number: PCT/AU99/00931 (22) International Filing Date: 27 October 1999 (27.10.99) (30) Priority Data: PP 6748 27 October 1998 (27.10.98) AU (71) Applicants (for all designated States except US): THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH [AU/AU]; Royal Parade, Parkville, Victoria 3052 (AU). THE COUNCIL FOR THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH [AU/AU]; 300 Herston Road, Herston, Queensland 4006 (AU). THE UNIVERSITY OF MELBOURNE [AU/AU]; Grattan Street, Parkville, Victoria 3052 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): BARTLETT, Perry, F. [AU/AU]; Walter and Eliza Hall Institute of Medical Research, Royal Parade, Parkville, Victoria 3052 (AU). HARTLEY, Lynne [AU/AU]; Walter and Eliza Hall Institute of Medical Research, Royal Parade, Parkville, Victoria 3052 (AU). PILIZZOTTO, Mark [AU/AU]; Walter and Eliza Hall Institute of Medical Research, Royal Parade, Parkville, Victoria 3052 (AU). KILPATRICK, Trevor [AU/AU]; Walter and Eliza Hall Institute of Medical Research, Royal Parade,		Parkville, Victoria 3052 (AU). KONTGEN, Frank [DE/AU]; 5 Timberglades, Park Orchards, Victoria 3114 (AU). COONAN, Jason [AU/AU]; Unit 10, 831 Park Street, Brunswick, Victoria 3056 (AU). GREFERATH, Ursula [DE/AU]; 366 Cardigan Street, Carlton, Victoria 3053 (AU). BOYD, Andrew, W. [AU/AU]; Queensland Institute of Medical Research, 300 Herston Road, Herston, Queensland 4006 (AU). DOTTORI, Mirella [AU/AU]; Queensland Institute of Medical Research, 300 Herston Road, Herston, Queensland 4006 (AU). GALEA, Mary [AU/AU]; University of Melbourne, Grattan Street, Parkville, Victoria 3052 (AU). PAXINOS, George [AU/AU]; The University of Melbourne, Grattan Street, Parkville, Victoria 3052 (AU). MURPHY, Mark [AU/AU]; The University of Melbourne, Grattan Street, Parkville, Victoria 3052 (AU). (74) Agents: HUGHES, E., John, L.; Davies Collison Cave, 303 Coronation Drive, Milton, Queensland 4064 (AU) et al. (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: A METHOD OF TREATMENT (57) Abstract <p>The present invention relates generally to a method of treatment and in particular a method of treating disorders of the nervous system such as arising from or during disease or injury. The method of the present invention involves manipulating expression of Eph receptors or their functional equivalents to increase or decrease expression or function depending on the condition being treated.</p>		

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: (other than US) THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH, COUNCIL FOR THE QUEENSLAND INSTITUTE FOR MEDICAL RESEARCH LIMITED, UNIVERSITY OF MELBOURNE
(US only) Perry F BARTLETT, Andrew W BOYD, Mirella DOTTORI, Lynne HARTLEY, Mary GALEA, George PAXINOS, Mark POLIZZOTTO, Trevor KILPATRICK, Mark MURPHY, Frank KONTGEN, Jason COONAN, Ursula GREFERATH

(ii) TITLE OF INVENTION: A METHOD OF TREATMENT

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: INTERNATIONAL (PCT)
(B) FILING DATE: 27-OCT-1999
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: AUSTRALIAN PROVISIONAL
(B) FILING DATE: 27-OCT-1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: HUGHES, DR E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/AF

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777

(B) TELEFAX: +61 3 9254 2770

(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotides

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGTGCTACTT CCATTGTGCA CGTCCTG

27

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotides

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCCGTGATA GCAAATTTGA G

21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotides

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGGAAGTGAG CATTATGGAT GA

22

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotides

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCTCCTCGT GCCCAGCGTT

20

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Oligonucleotides

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTAGAATTCC CCGAGGAGGA GCTGTAC

27

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: Oligonucleotides

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTAGAATTCT GCAGTCCCAC CACCCCG

27

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00931

A. CLASSIFICATION OF SUBJECT MATTERInt Cl⁶:

A61K 38/16, 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K WITH ELECTRONIC DATABASE AS INDICATED BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU : IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLU/ MEDLINE/ WPAT:

1) EPHRIN OR SEK 1 OR EPHA4 2) 1 AND AXON 3) 1 AND NEUR 4) 1 AND NERV 5) 2 OR 3 OR 4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO-A-96/09384 (President & Fellows of Harvard College) 28 March 1996 - partic pages 47-51	1-10,12, 18, 20, 21 11,13,14-17, 19
X Y	WO-A- 96/23000 (Amgen Inc) 1 August 1996 - in partic page 16 claims 30, 31	1-10,12, 18, 20,21 11,13, 14-17, 19
X Y	WO 96/13518 (Genentech) 9 May 1996 - in partic pages 22-25, claims 24, 25	1-10, 12, 18, 20, 21 11,13,14-17, 19

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	Document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent but published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

27 November 1999

Date of mailing of the international search report

17 DEC 1999

Name and mailing address of the ISA/AU

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Authorized officer

JENNIFER FERNANCE
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00931

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 96/26958 (President & Fellows of Harvard College) 6 September 1996 -in part pages 25-26, claims 40-48, 58.	1-10,12,18,20,21 11,13,14-17,19
X Y	WO 97/04091 (Amrad Operations Pty Ltd) 6 February 1997 - in part page 24.	1-12, 18, 20,21 13, 14-17, 19
X Y	WO 97/14966 (Mt Sinai Hospital Corporation) 24 April 1997 - in part pages 3, 17, 18.	1-10, 12, 14-18, 20,21 11, 13,19
X Y	EMBO Journal, Vol 16 No11 (June 1997), pages 3106-3114, "Aberrant Axonal Projections in Mice Lacking EphA8 (Eek) Tyrosine Protein Kinases Receptors", Park et al	14-17 1-13, 18-21
Y	Curr. Biol, Vol 7 No 8 (August 1997). Pages 561-570, "The EphA4 and EphB1 Receptor tyrosine Kinases and Ephrin-B2 Ligand regulate targeted Migration of Branchial Neural Crest Cells", Smith et al	1-21
Y	Exercise and Sport Science Reviews, Vol 22 (1994) pages 361-388, "Transgenic Animal Models", Tsika.	1-21
X Y	Cur. Opin. Neuro., Vol 8 No 1 (February 1998) pages 117-127, "Cell-Contact-Dependent Signalling in Axon Growth and Guidance: Eph Receptor Tyrosine Kinases and Receptor Protein tyrosine phosphatase β ", Holland et al.	14-17 1-13, 18-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU 99/00931

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	9623000	AU	49047/96	CA	2210644		
WO	9704091	AU	64098/96	EP	842272		
WO	9613518	AU	44053/96	CA	2203888	EP	787148
WO	9609384	AU	35920/95	US	5795734		
END OF ANNEX							

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT

(PCT Article 36 and Rule 70)

REC'D 13 FEB 2001

15

Applicant's or agent's file reference 2211510	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).	
International Application No. PCT/AU99/00931	International Filing Date (day/month/year) 27 October 1999	Priority Date (day/month/year) 27 October 1998
International Patent Classification (IPC) or national classification and IPC Int. Cl. ⁷ A61K 38/16, 38/00 A61P 25/00, 25/14, 25/28		
Applicant The Walter and Eliza Hall Institute of Medical Research (et al)		

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 5 sheets, including this cover sheet.
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

- | | | |
|------|-------------------------------------|---|
| I | <input checked="" type="checkbox"/> | Basis of the report |
| II | <input type="checkbox"/> | Priority |
| III | <input type="checkbox"/> | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | <input type="checkbox"/> | Lack of unity of invention |
| V | <input checked="" type="checkbox"/> | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | <input type="checkbox"/> | Certain documents cited |
| VII | <input type="checkbox"/> | Certain defects in the international application |
| VIII | <input checked="" type="checkbox"/> | Certain observations on the international application |

Date of submission of the demand 17 May 2000	Date of completion of the report 12 January 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer JENNIFER FERNANCE Telephone No. (02) 6283 2416

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed.
- ☐ the description, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the claims, pages , as originally filed,
 pages , as amended (together with any statement) under Article 19,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the drawings, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the sequence listing part of the description:
 pages , as originally filed
 pages , filed with the demand
 pages , received on with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, was on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims 11, 12	YES
	Claims 1-10, 13-21	NO
Inventive step (IS)	Claims 11, 12	YES
	Claims 1-10, 13-21	NO
Industrial applicability (IA)	Claims 14-21	YES
	Claims 1-13	NO

2. Citations and explanations (Rule 70.7)Documents

- 1) WO-A-96/09384
- 2) WO-A-96/23000
- 3) WO-A-96/13581
- 4) WO-A-96/26958
- 5) WO-A-97/04091
- 6) WO-A-97/14966
- 7) Park et al
- 8) Smith et al
- 9) Tsika
- 10) Holland et al

Novelty (N) Claims 1-10, 13-21

In light of comments made in Box VIII, documents 1-7 disclose the method and composition claims, claims 1-10, 13 and 18-21, wherein the ligands and receptors include the ephrins Elf 1, HEK 4, AL-1, Elf 2, LERK 4, EphA8, Elk-L, Ehk 1, B61; sek 1 compositions are also disclosed as known.

Documents 7, 8 and 10 disclose the transgenic animals as claimed in claims 14-17.

Documents 1)-10) do not disclose the methods of claims 11 and 12. Therefore these claims are considered novel (see comments made in Box VIII).

Inventive Step (IS) Claims 1-10, 13-21

Claims 1-10, 13-21 as above.

It would not be obvious to the Skilled Addressee from documents 1)-10), alone or in different combinations, to perform the invention of claims 11 and 12. Thus claims 11 and 12 are considered to be inventive (see comments in Box VIII).

(Continued in Supplemental Box).

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 11 and 12 are not supported by the description. There is no disclosure or support for these claims within the description.

Examination has been undertaken on the understanding that:

- i) an "Eph receptor or its functional equivalent" comprises a member of the Eph subfamily;
- ii) an ephrin is a ligand which binds to a receptor of the Eph family, for example, LERK 4, B61, Elf 1 and Elk-L;
- iii) EphA4 is sek 1 (noted in the specification at page 1) and sek 1 is known as sek.

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

C ntinuation of Box VIndustrial Applicability Claims 1-13

Claims 1-13 are to methods of treatment and are subject to rule 67.1 and, as such, do not require an International Preliminary Examination. However, as they do not contravene Australian law, they have been examined

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2211510	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU99/00931	International Filing Date (<i>day/month/year</i>) 27 October 1999	Priority Date (<i>day/month/year</i>) 27 October 1998
International Patent Classification (IPC) or national classification and IPC Int. Cl. ⁷ A61K 38/16, 38/00 A61P 25/00, 25/14, 25/28		
Applicant The Walter and Eliza Hall Institute of Medical Research (et al)		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.																								
2.	This REPORT consists of a total of 5 sheets, including this cover sheet. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of sheet(s).																								
3.	This report contains indications relating to the following items: <table style="width: 100%; border: none;"><tr><td style="width: 5%;">I</td><td style="width: 5%; text-align: center;"><input checked="" type="checkbox"/></td><td>Basis of the report</td></tr><tr><td>II</td><td style="text-align: center;"><input type="checkbox"/></td><td>Priority</td></tr><tr><td>III</td><td style="text-align: center;"><input type="checkbox"/></td><td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td></tr><tr><td>IV</td><td style="text-align: center;"><input type="checkbox"/></td><td>Lack of unity of invention</td></tr><tr><td>V</td><td style="text-align: center;"><input checked="" type="checkbox"/></td><td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td></tr><tr><td>VI</td><td style="text-align: center;"><input type="checkbox"/></td><td>Certain documents cited</td></tr><tr><td>VII</td><td style="text-align: center;"><input type="checkbox"/></td><td>Certain defects in the international application</td></tr><tr><td>VIII</td><td style="text-align: center;"><input checked="" type="checkbox"/></td><td>Certain observations on the international application</td></tr></table>	I	<input checked="" type="checkbox"/>	Basis of the report	II	<input type="checkbox"/>	Priority	III	<input type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/>	Lack of unity of invention	V	<input checked="" type="checkbox"/>	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input type="checkbox"/>	Certain documents cited	VII	<input type="checkbox"/>	Certain defects in the international application	VIII	<input checked="" type="checkbox"/>	Certain observations on the international application
I	<input checked="" type="checkbox"/>	Basis of the report																							
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III	<input type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability																							
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VI	<input type="checkbox"/>	Certain documents cited																							
VII	<input type="checkbox"/>	Certain defects in the international application																							
VIII	<input checked="" type="checkbox"/>	Certain observations on the international application																							

Date of submission of the demand 17 May 2000	Date of completion of the report 12 January 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer JENNIFER FERNANCE Telephone No. (02) 6283 2416

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed.
- ☐ the description, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the claims, pages , as originally filed,
 pages , as amended (together with any statement) under Article 19,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the drawings, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the sequence listing part of the description:
 pages , as originally filed
 pages , filed with the demand
 pages , received on with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, was on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims 11, 12	YES
	Claims 1-10, 13-21	NO
Inventive step (IS)	Claims 11, 12	YES
	Claims 1-10, 13-21	NO
Industrial applicability (IA)	Claims 14-21	YES
	Claims 1-13	NO

2. Citations and explanations (Rule 70.7)Documents

- 1) WO-A-96/09384
- 2) WO-A-96/23000
- 3) WO-A-96/13581
- 4) WO-A-96/26958
- 5) WO-A-97/04091
- 6) WO-A-97/14966
- 7) Park et al
- 8) Smith et al
- 9) Tsika
- 10) Holland et al

Novelty (N) Claims 1-10, 13-21

In light of comments made in Box VIII, documents 1-7 disclose the method and composition claims, claims 1-10, 13 and 18-21, wherein the ligands and receptors include the ephrins Elf 1, HEK 4, AL-1, Elf 2, LERK 4, EphA8, Elk-L, Ehk 1, B61; sek 1 compositions are also disclosed as known.

Documents 7, 8 and 10 disclose the transgenic animals as claimed in claims 14-17.

Documents 1)-10) do not disclose the methods of claims 11 and 12. Therefore these claims are considered novel (see comments made in Box VIII).

Inventive Step (IS) Claims 1-10, 13-21

Claims 1-10, 13-21 as above.

It would not be obvious to the Skilled Addressee from documents 1)-10), alone or in different combinations, to perform the invention of claims 11 and 12. Thus claims 11 and 12 are considered to be inventive (see comments in Box VIII).

(Continued in Supplemental Box).

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 11 and 12 are not supported by the description. There is no disclosure or support for these claims within the description.

Examination has been undertaken on the understanding that:

- i) an "Eph receptor or its functional equivalent" comprises a member of the Eph subfamily;
- ii) an ephrin is a ligand which binds to a receptor of the Eph family, for example, LERK 4, B61, Elf 1 and Elk-L;
- iii) EphA4 is sek 1 (noted in the specification at page 1) and sek 1 is known as sek.

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box VIndustrial Applicability Claims 1-13

Claims 1-13 are to methods of treatment and are subject to rule 67.1 and, as such, do not require an International Preliminary Examination. However, as they do not contravene Australian law, they have been examined

PATENT COOPERATION TREATY

BRISBANE PCT

THURSDAY 18 MAY 2000

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

HUGHES, E., John, L.
Davies Collison Cave
303 Coronation Drive
Milton, Queensland 4064
AUSTRALIE

Date of mailing (day/month/year) 04 May 2000 (04.05.00)		IMPORTANT NOTICE	
Applicant's or agent's file reference 2211510			
International application No. PCT/AU99/00931	International filing date (day/month/year) 27 October 1999 (27.10.99)	Priority date (day/month/year) 27 October 1998 (27.10.98)	
Applicant THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,CN,JP,KP,KR,MA,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,
GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,
PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
04 May 2000 (04.05.00) under No. WO 00/24413

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des C. Iombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740.14.35	Authorized officer J. Zahra Telephone No. (41-22) 338.83.38
---	---

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 June 2000 (09.06.00)	
International application No. PCT/AU99/00931	Applicant's or agent's file reference 2211510
International filing date (day/month/year) 27 October 1999 (27.10.99)	Priority date (day/month/year) 27 October 1998 (27.10.98)
Applicant BARTLETT, Perry, F. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

17 May 2000 (17.05.00)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer C. Cupello Telephone No.: (41-22) 338.83.38
--	---

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year) 09 June 2000 (09.06.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 2211510	
International application No. PCT/AU99/00931	International filing date (day/month/year) 27 October 1999 (27.10.99)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address HUGHES, E., John, L. Davies Collison Cave 303 Coronation Drive Milton, Queensland 4064 Australia	State of Nationality	State of Residence
	Telephone No. +61 7 3368 2255	
	Facsimile No. +61 7 3368 2262	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address HUGHES, E., John, L. Davies Collison Cave 1 Little Collins Street Melbourne, VIC 3000 Australia	State of Nationality	State of Residence
	Telephone No. +61 3 9254 2777	
	Facsimile No. +61 3 9254 2770	
	Teleprinter No.	

3. Further observations, if necessary:

The new agent's address on the Demand has been considered as a change under Rule 92bis. In case of disagreement, the International Bureau should be notified immediately.

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer C. Cupello Telephone No.: (41-22) 338.83.38
---	--

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

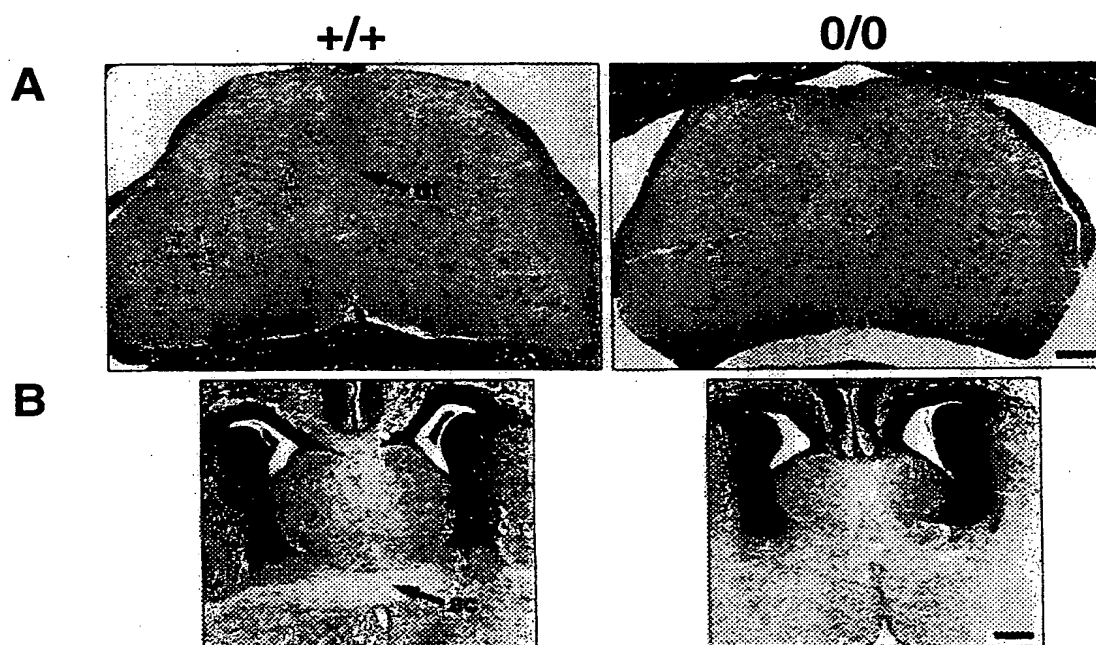
HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year) 18 April 2001 (18.04.01)	
Applicant's or agent's file reference 2211510	IMPORTANT NOTIFICATION
International application No. PCT/AU99/00931	International filing date (day/month/year) 27 October 1999 (27.10.99)

1. The following indications appeared on record concerning: <input checked="" type="checkbox"/> the applicant <input type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative		
Name and Address COUNCIL FOR THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH 300 Herston Road Herston, Queensland 4006 Australia	State of Nationality AU	State of Residence AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: <input type="checkbox"/> the person <input checked="" type="checkbox"/> the name <input type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence		
Name and Address THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH 300 Herston Road Herston, Queensland 4006 Australia	State of Nationality AU	State of Residence AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to: <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> the receiving Office <input type="checkbox"/> the International Searching Authority <input type="checkbox"/> the International Preliminary Examining Authority </div> <div> <input type="checkbox"/> the designated Offices concerned <input checked="" type="checkbox"/> the elected Offices concerned <input type="checkbox"/> other: </div> </div>		

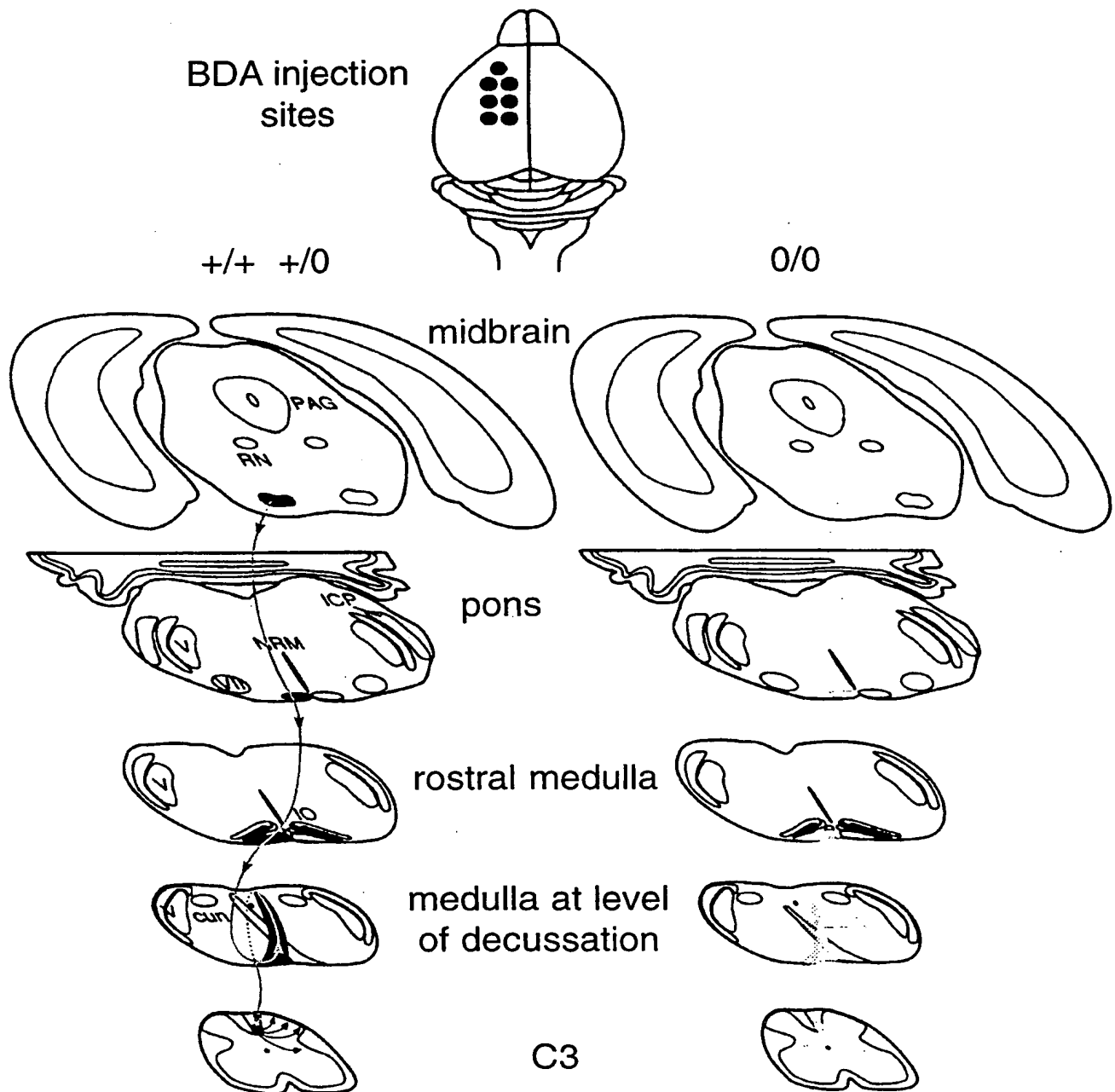
CORRECTED
VERSION

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Céline Faust Telephone No.: (41-22) 338.83.38
--	---



3/8

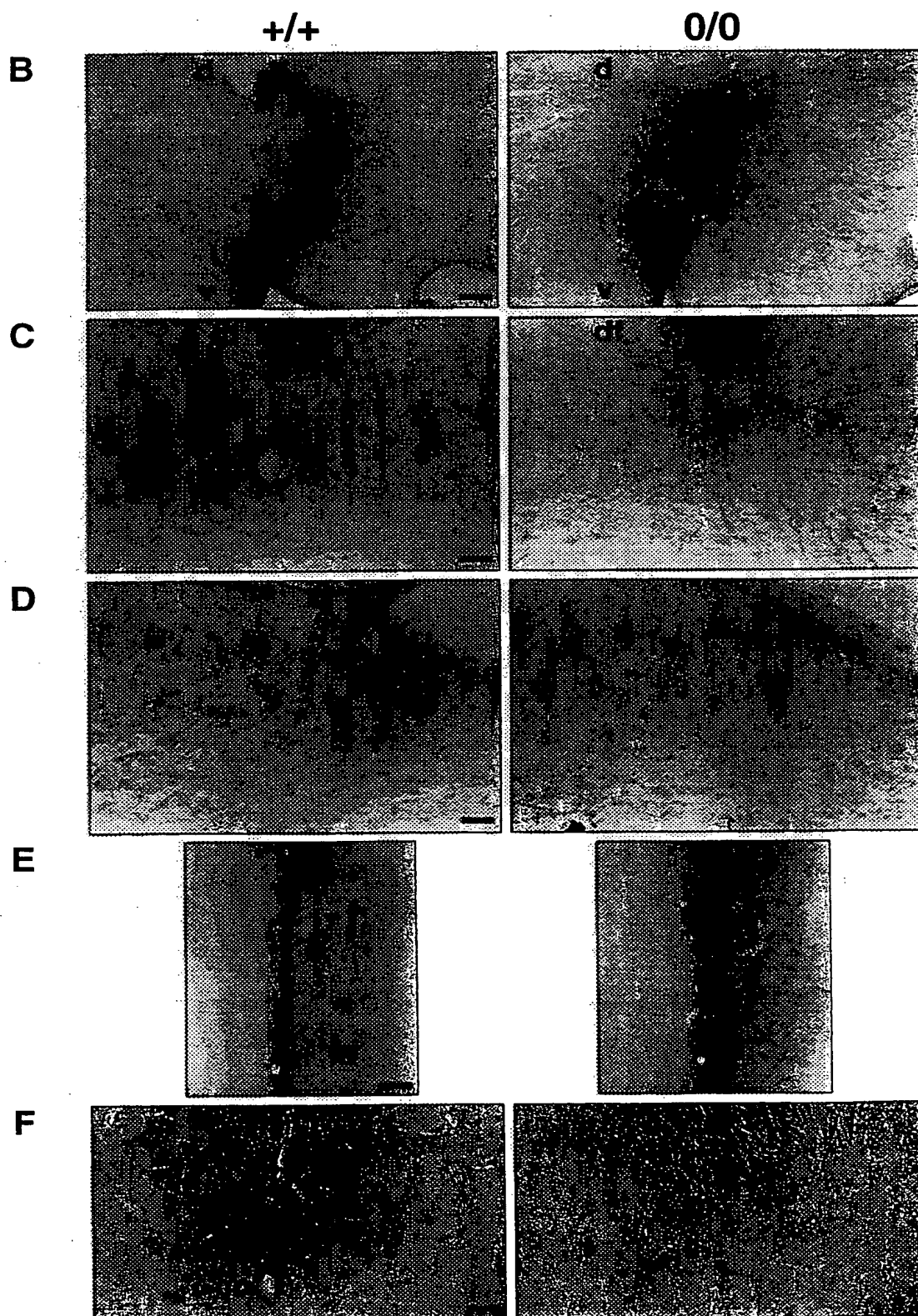
Corticospinal projections in the mouse



Legend:

PAG - periaqueductal grey
 ICP - inferior cerebellar peduncle
 V - trigeminal nucleus
 IO - inferior olive

RN - red nucleus
 NRM - nucleus raphe magnus
 VII - facial nucleus
 cun - cuneate nucleus



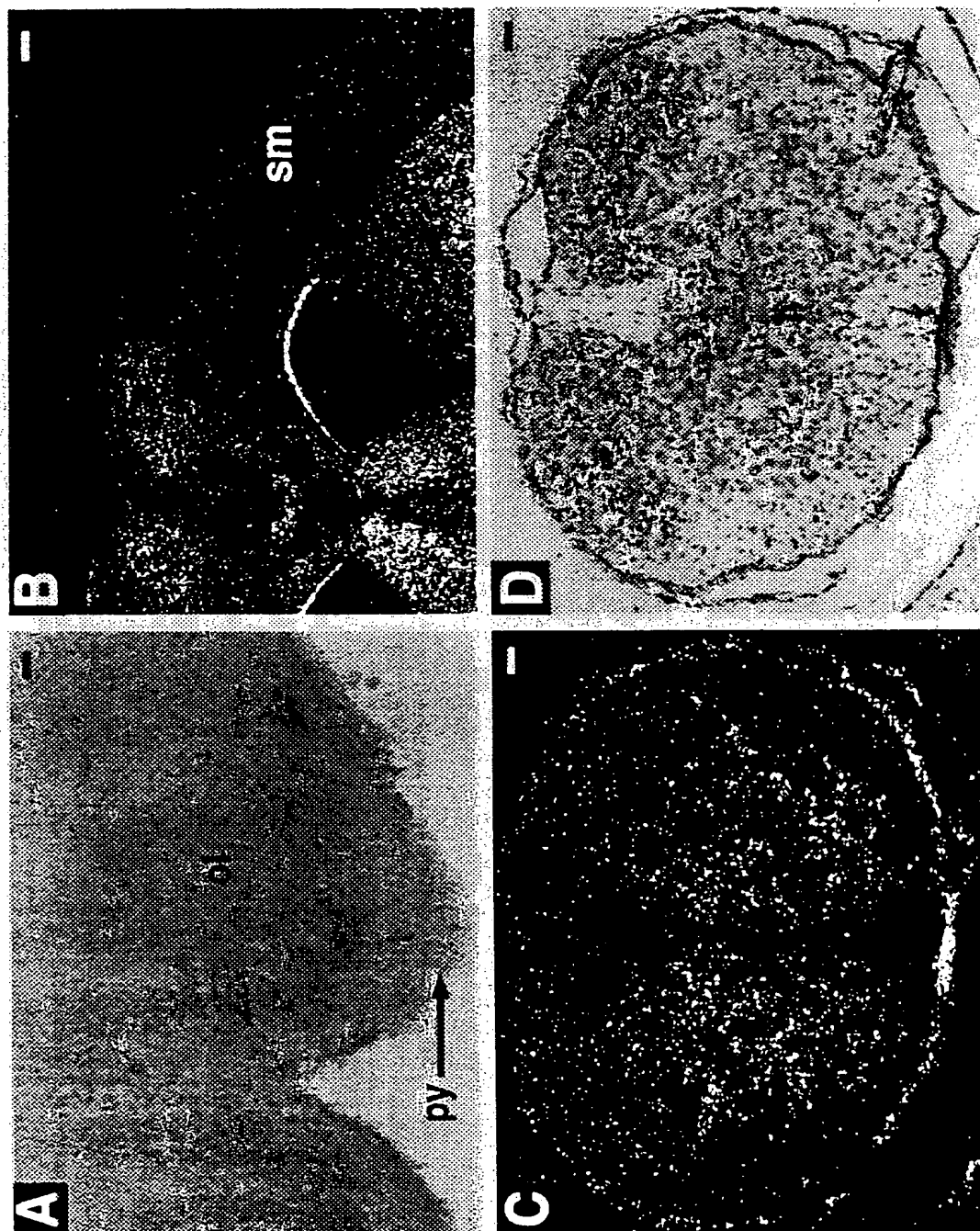


Figure 4

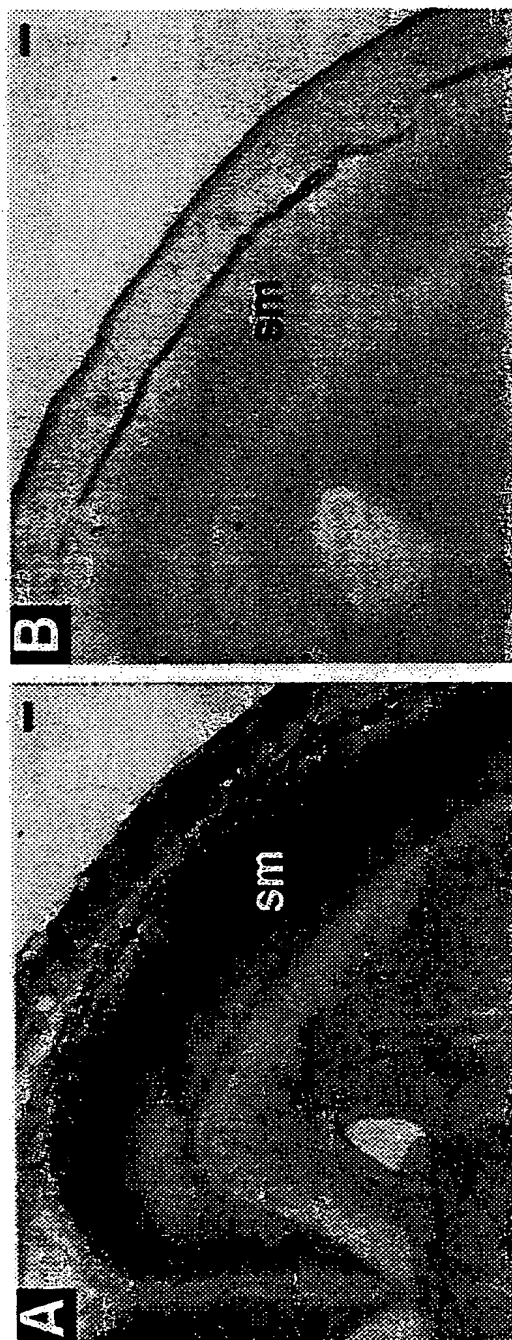
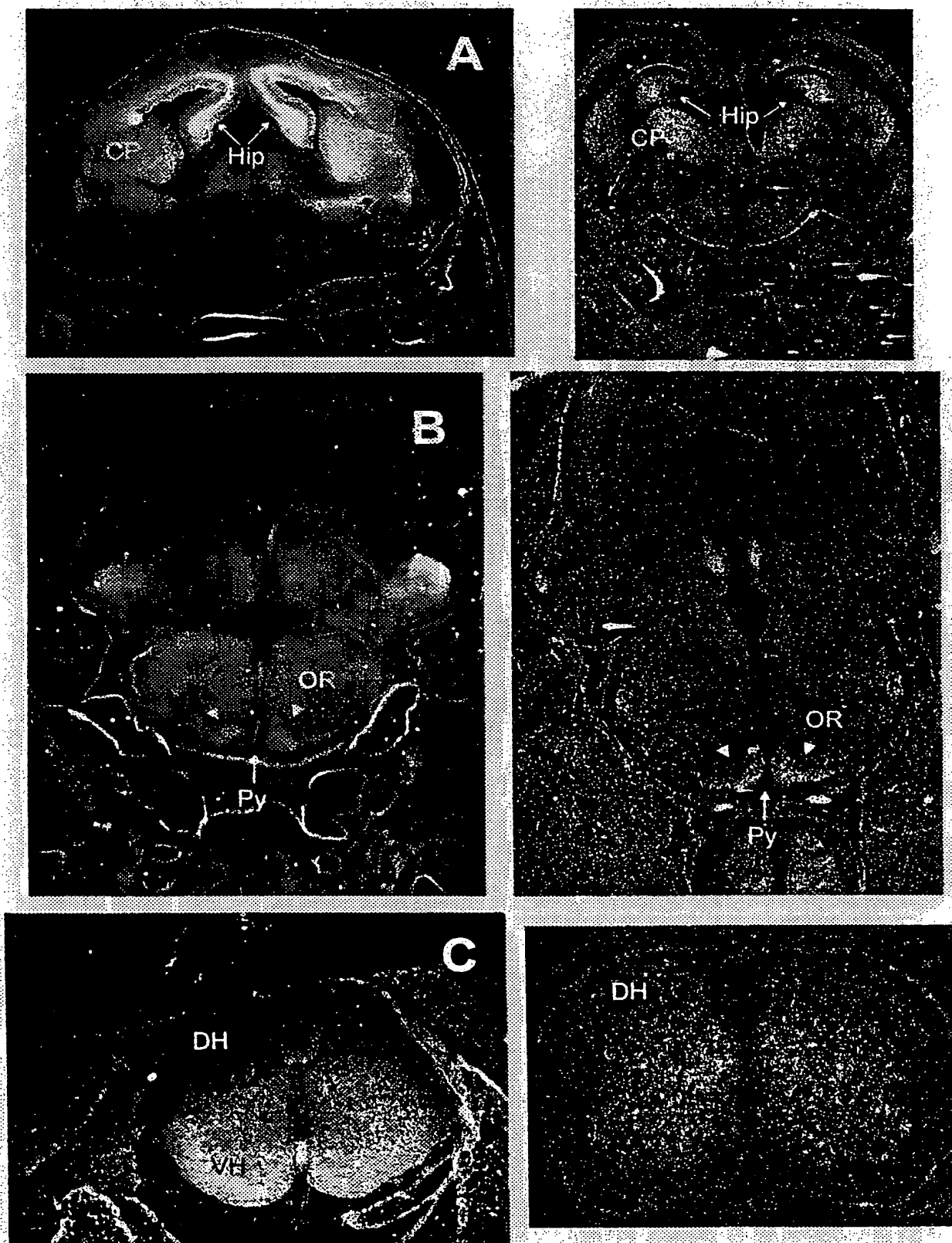


Figure 5



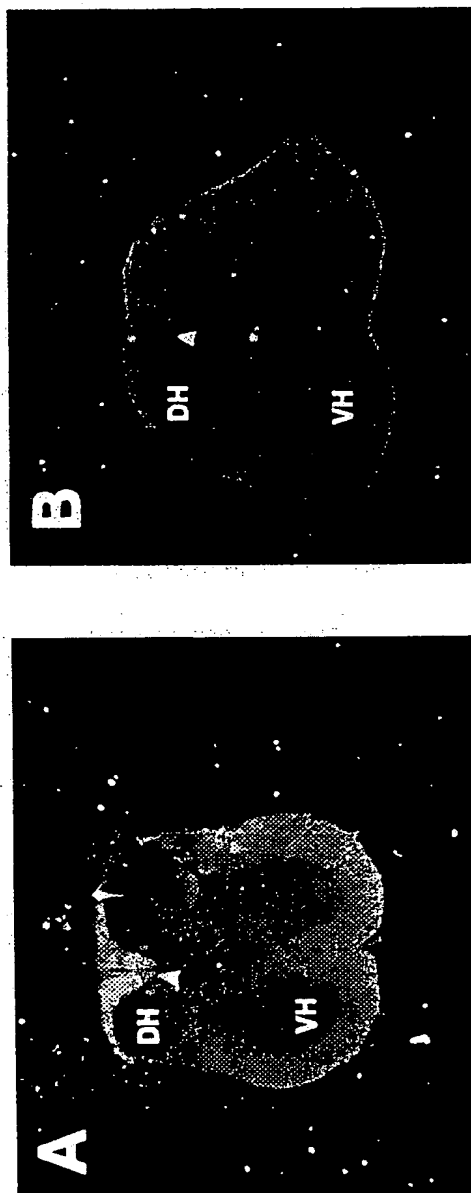


Figure 7

A METHOD OF TREATMENT

The present invention relates generally to a method of treatment and in particular a method of treating disorders of the nervous system such as arising from or during disease or injury.

5 The method of the present invention involves manipulating expression of Eph receptors or their functional equivalents to increase or decrease expression or function depending on the condition being treated.

Bibliographic details of the publications numerically referred to in this specification are
10 collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Recent studies show that axons are guided to their targets by a system of guidance molecules
15 including Eph receptors and their ligands (1-3). The role of these molecules has been intensely studied in development of the visual system (4-6), where the reciprocal gradient expression of the Eph receptors in the retina and of their ligands in the optic tectum is the suggested basis for the formation of the retinotectal topographic map. Other observations pertinent to the role of these molecules in the developing nervous system include axonal fasciculation and establishing
20 brain commissures (7-9).

The Eph family of receptors can be divided into two groups, EphA and EphB, based on the sequence similarities of their extracellular domain (10). Each EphA receptor is able to bind several Ephrin A ligands which are associated with the membrane *via* a GPI-linkage, these
25 receptors show little or no binding to the transmembrane Ephrin B ligands (11, 12). The EphB group of receptors show the reverse pattern, binding predominantly to Ephrin B ligands. An exception to this 'rule' is the EphA4 (previously known as Sek1) receptor which was found to significantly bind to some of the transmembrane ligands in addition to all the GPI-linked ligands (11-13).

30

EphA4 expression during development shows a defined spatio-temporal pattern within the

- 2 -

developing forebrain, hindbrain and mesoderm (14, 15). In the final stages of embryogenesis, expression of EphA4 is predominantly found within regions of the central nervous system, including the cerebral cortex, striatum, thalamus, hippocampus, and ventral spinal cord. In the hindbrain, EphA4 shows restricted expression to rhombomeres 3 and 5 (14) which suggested
5 a role of this receptor in establishing boundaries during embryogenesis. This notion was supported by over expression of dominant negative, truncated EphA4 receptor in zebrafish embryos. The resultant mutant embryos were found to have disruption in the rhombomere boundaries and an expansion of the developing retina into the diencephalon (16, 17).

10 In work leading up to the present invention, the inventors generated laboratory animals deficient in the EphA4 receptor. The EphA4 mutant animals displayed a gross motor abnormality in the hindlimbs. Anatomical analyses and anterograde tracing of cortical neurons demonstrated a severe disruption of the corticospinal tract (CST) in these animals. The CST is the single longest axonal projection in the mammalian central nervous system (18). CST neurons arise
15 from layer V in the neocortex and extend their axons through the forebrain, midbrain and hindbrain, and terminate at various levels of the spinal cord. In primates the CST axons predominantly synapse directly with the spinal motor neurons, whereas in the rodent most of the cortical axons synapse with interneurons which then connect to the spinal motor neurons. The EphA4 null mutant animals showed specific defects in the CST both at the level of the
20 medulla and the spinal cord, which indicates that EphA4 is required for the correct formation of the CST.

Accordingly, one aspect of the present invention contemplates a method of facilitating regeneration, growth and/or development of a central nervous system and in particular the
25 central nervous system in a human or non-human animal said method comprising increasing, elevating or otherwise enhancing the levels of a Eph receptor or its functional equivalent.

Another aspect of the present invention provides a method of regulating axon guidance in a human or non-human animal said method comprising increasing, elevating or otherwise
30 enhancing the levels of an Eph receptor or its functional equivalent in said human or non-human animal.

- 3 -

Still another aspect of the present invention is directed to a method for facilitating the repair or replacement of axons in a human or non-human animal, said method comprising increasing, elevating or otherwise enhancing the levels of an Eph receptor or its functional equivalent in a region surrounding the cortex and/or inhibiting, reducing or otherwise down-regulating expression of the Eph receptor or its functional equivalent when expressed in tissues outside said region surrounding the cortex and which expression leads to blockage of axonal growth.

Yet another aspect of the present invention provides for a method of inducing, promoting or otherwise facilitating repair of nervous tissue in a human or non-human animal, said method comprising increasing, elevating or otherwise enhancing the levels of an Eph receptor or its functional equivalent in a region surrounding the cortex and/or inhibiting, reducing or otherwise down-regulating expression of the Eph receptor or its functional equivalent when expressed in tissues outside said region surrounding the cortex and which expression leads to blockage of axonal growth.

The repair of nervous tissue according to this aspect of the present invention may be required following or during disease or trauma. Particular diseases contemplated by the present invention include but are not limited to brain and spinal cord injury, diseases of the upper motor neuron and diseases of the central nervous system such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.

The present invention may also be practiced by modulating levels of the ligands for Eph receptors or their functional equivalents, e.g. the ephrins or their functional equivalents.

Particularly preferred ephrins include ephrin-B1, ephrin-B2 and ephrin-B3. The most preferred ephrin is ephrin-B3.

The method of the present invention may be accomplished in any number of ways including but not limited to administering soluble or near soluble forms of the Eph receptors or parts

- 4 -

thereof (e.g. fragment comprising all or part of the extracellular domain) or their functional equivalents in monomeric, dimeric or other multimeric form. Administration may be in any convenient means such as directly into the spinal cord or brain. Since it is proposed, in accordance with the present invention, that the Eph receptors or their functional equivalents
5 regulate axon guidance in the corticospinal tract (CST), the administration of a monomeric or multimeric form of the Eph receptors or parts thereof or ligands or their functional equivalents may assist in defining pathways for axon movement.

Where Eph receptors or their functional equivalents are expressed in inappropriate tissues,
10 i.e. not in the region surrounding the CST, then soluble forms of ephersins or other Eph antagonists may be administered, such as to the brain and/or spinal cord, to block the expression or function of the Eph receptors or their functional equivalents. An example of other Eph antagonists include receptor monomers or other derivatives or their functional equivalents. Labelled Eph monomers such as FLAG-tagged Eph monomers (40, 41) are
15 particularly useful antagonists.

Reference herein to "Eph receptor" means the murine Eph receptor or a functional equivalent thereof such as a human or non-murine homologue. The present invention further extends to the manipulation of derivatives of the Eph receptor or its functional equivalent. A
20 derivative includes a part, fragment or portion of the receptor such as a single or multiple amino acid substitution, deletion and/or addition to the amino acid sequence defining the Eph receptor or its functional equivalent. The present invention further extends to ligand binding portions of the Eph receptor such as an extracellular portion of the receptor. An example of a fragment of an Eph receptor having ligand binding capacity is US Patent Application No.
25 09/104,340 entitled "Receptor ligand system and assay".

Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to an Eph receptor or its functional equivalent or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding an Eph receptor or its functional
30 equivalent. "Additions" to amino acid sequences or nucleotide sequences include fusions with

- 5 -

other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to an Eph receptor includes reference to all derivatives thereof including functional and non-functional derivatives as well as homologues and analogues thereof.

- 5 Analogues of an Eph receptor contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.
- 10 Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with
- 15 succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

20

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

- Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic
- 25 acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

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Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

5

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

15

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

25

The present invention further contemplates chemical analogues of an Eph receptor capable of acting as antagonists or agonists of an Eph receptor or which can act as functional analogues of an Eph receptor. Chemical analogues may not necessarily be derived from an Eph receptor but may share certain conformational similarities. Alternatively, chemical analogues may be

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specifically designed to mimic certain physiochemical properties of an Eph receptor. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

- 5 These types of modifications may be important to stabilise an Eph receptor if administered to an individual or for use as a diagnostic reagent.

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule.

- 10 Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5 α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
10 aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgln
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15 D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Das	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30 D-threonine	Dthr	L-norleucine	Nle

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	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
5	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpn
	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
10	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
15	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
20	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
25	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
30	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser

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	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methyllleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
5	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
10	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
15	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
20	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methyllleucine	Mleu	L- α -methyllysine	Mlys
	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
25	L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
	L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
	L- α -methylserine	Mser	L- α -methylthreonine	Mthr
	L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
	L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhph

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N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
ethylamino)cyclopropane			

5

The present invention further contemplates modified animals with altered expression levels of an Eph receptor or its functional equivalent. Such modified animals include "knock-out" murine animals such as "knock-out" mice. Alternatively, the modified animals have

10 increased expression levels of the Eph receptor or its functional equivalent or expression in particular tissue or targeting expression in a region surrounding the CST.

The preferred Eph receptor is EphA4 or its functional equivalent in murine species or non-murine species (e.g. humans).

15

The present invention further extends to agonists and antagonists of an Eph receptor such as EphA4 or its functional equivalent and pharmaceutical compositions comprising same. The present invention also extends to genetic molecules encoding the Eph receptor or its functional equivalent or encoding an agonist, antagonist or ligand thereof. Particularly

20 useful antagonists comprise monomeric Eph receptor molecules or their functional equivalents, soluble forms of the Eph receptor ligands (e.g. epherins) or molecules detected following screening of natural product or chemical libraries. Particularly useful epherins include epherin B3 and EphA4-binding epherins.

25 The use of the expression of the Eph receptor to guide axonal movement has therapeutic implications including the use of Eph receptors to direct therapeutic molecules to particular targets.

The present invention is now further described with reference to the preferred Eph receptor,

30 EphA4 and to a "knock-out" mouse for the EphA4 gene. This is done, however, with the

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understanding that the present invention extends to any Eph receptor or its functional equivalent which is involved in axonal guidance in humans or non-human animals. Reference to non-human animals include livestock animals (e.g. sheep, horses, pigs, donkeys, cows), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters), companion
5 animals (e.g. dogs, cats) and captured wild animals.

The EphA4 null (i.e. "knock-out") mutant mice are the first Eph receptor null mice to display a motor phenotype (7-9). This motor defect is more marked in the hindlimbs and the animals have an abnormal 'hopping' gait. Analysis of the CST in these animals reveal a reduced
10 number of CST axons in the lower spinal cord segments and an abnormal pattern of termination at higher segments of the spinal cord and medulla. This progressive diminution of the CST, relative to normal animals, along the length of the cord is consistent with the more marked motor defect observed in the lower limbs of these animals. Additionally, it has been observed that some rats which have had their CST disrupted by transection also show a
15 phenotype with a hopping gait similar to the EphA4 null mutants. Thus, a defective CST accounts for the motor defect.

The perturbation of the CST in null mutant animals establishes that EphA4 is required for CST development. During CST development, the first pioneering axons to advance down the spinal
20 cord are those that will innervate the lumbar segments and these are then followed by a bulk of later arriving fasciculating CST fibres projecting to upper cord segments (18). As the primary growth cones of corticospinal axons continue to elongate down the midline of the spinal cord, the brainstem and spinal cord targets are contacted by collateral branches sprouted along the corticospinal axon shafts (32). The paucity of CST axons observed within the
25 lumbar spinal cord regions in the EphA4 mutants are presumably due to misguidance of the primary cortical axons. It is possible that guidance of the collateral branches along the whole CST are also disrupted. Altogether, these data strongly indicate that EphA4 regulates axon guidance in the CST.

30 The immunohistochemistry and *in situ* hybridization data suggest that EphA4 is not expressed by cortical motor neurons or in the CST during its development. However, EphA4 was found

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highly expressed within the intermediate and ventral regions of the spinal cord which is the region where the CST axons do not normally terminate. This is consistent with the notion that EphA4 is expressed on structures surrounding the CST where it acts as a signal for CST axons bearing Ephrin ligands to be appropriately guided. Also consistent with this model, EphrinB3 mRNA was detected within the sensorimotor cortex at E18.5 which suggests that this transmembrane ligand is expressed on CST axons as they extend through the brain and spinal cord. EphA4 binds to EphrinB3 with high affinity and the transmembrane Ephrin ligands have been shown to induce signalling upon receptor binding (12, 13, 33, 34).

Both *in vitro* and *in vivo* studies have suggested that the Eph receptor family regulate axon guidance through mechanisms of contact repulsion rather than attraction (5, 6, 35, 36). For example, in EphB2 receptor-null mice the posterior tract of the AC innervates the floor of the brain aberrantly (7). EphB2 is normally expressed in areas ventral to the commissure and the commissural axons express a ligand for EphB2, Ephrin-B1. This suggests, therefore, that EphB2 repels AC axons from entering this ventral area via Ephrin-mediated signals (33, 34). The present invention is consistent with a similar mechanism relating to guidance of the CST.

Another molecule found to be involved in CST development is the neural cell adhesion molecule, L1. In mice deficient in L1 many of the CST axons failed to decussate at the medulla, passing ipsilaterally into the dorsal columns (31). Similar to EphA4 null mice, the number of CST axons within the dorsal funiculus of the spinal cord was reduced and these axons did not project beyond the cervical levels. It was proposed that the interaction of L1 on the axons with CD24 (expressed in the midline) may modify the CST axons response to midline inhibitory cues, thereby allowing the axons to cross the midline. Another molecule shown to act as a guidance cue for CST axons is Netrin-1 (37). It was shown that the pathfinding of CST axons from the cortex to the internal capsule of the forebrain may be mediated by the chemoattractive activity of Netrin-1. Although not intending to limit the present invention to any one theory or mode of action, it is proposed herein that CST axons are guided by the combined actions of a number of attractive and repulsive guidance cues.

30

The present invention provides *inter alia* an understanding of the role that EphA4 plays in

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mammalian neural development. The inventors show that EphA4 may not be required for initial corticospinal tract development and that it is also not required for adult pinal motoneuron morphological development and survival. However, the inventors do show that EphA4 plays an important role in the correct topographic positioning of some spinal cord
5 motoneuron populations. EphA4 is expressed in specific areas of the brain during late embryonic development. These data provide an explanation for the axonal abnormalities observed in the EphA4 null mutant. The inventors show that EphA4 is widely expressed in the adult spinal cord after traumatic injury and this may contributed to the lack of axonal regeneration observed following spinal cord trauma. This knowledge of EphA4 expression
10 after injury is important for the clinical treatment of spinal cord injury as well as other central nervous system diseases such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.

The present invention is further described by the following non-limiting Figures and
15 Examples.

In the Figures:

Figure 1 is a representation showing targeted disruption of EphA4 gene. (A) Partial map of
20 the *ephA4* genomic locus (+/+) with the targeting construct and the resulting targeted loci (o/o). The EphA4 targeting vector was designed to replace exon III (217bp-880bp of EphA4 cDNA) (38) with the 1.8kb neomycin selection gene. For homologous recombination, 5' *HindIII*-*SacI* 3kb sequence and 3' *Eco47III*-*BamHI* 5.5kb sequence flanking exon III were subcloned into the pKJ1 vector. Homologous recombination would cause a frame shift in the EphA4 gene
25 resulting in a null mutant protein (Fig. 4.2). The probe used for all Southern analysis was a 1kb genomic fragment containing exon II (149bp-216bp) and *EcoRI* site. Ec, *EcoRI*; H, *HindIII*; S, *SacI*; E47, *Eco47III*; B, *BamHI*; Neo, neomycin gene; II, exon II; III, exon III. (B) Genotype analysis of EphA4 homozygous (o/o), heterozygous (+/o), and wild type (+/+) animals. Genomic DNA was isolated from 0.5cm tail tissue (39), digested with *EcoRI* and
30 subjected to Southern blot analysis using the 5' external probe shown in A. Alleles bearing the *ephA4* mutation results in a 5kb band, whereas an 11kb band is observed in the wild type

alleles. (C) Whole-mount immunocytochemistry of E8.5 embryos using anti-EphA4 antibody. EphA4 is expressed in rhombomeres 3 and 5 (arrows) in heterozygotes (+/o), but no EphA4 protein is detected in homozygous (o/o) mutants. The embryos were genotyped by PCR from yolk sac DNA.

5

Figure 2 is a photographic representation of histological sections of EphA4 homozygous (o/o) and wild type (+/+) animals. (A) Transverse sections stained with luxol fast blue of lumbar spinal cord from adult mice. Area of the dorsal funiculus (df) appears to be shallower in EphA4 homozygotes. Scale bar = 160 μ m. (B) Coronal sections stained with haematoxylin and eosin of E16 embryo brains. A loss of the anterior commissure (ac) is observed in

Figure 3 shows labelled CST in normal (+/+ and +/o) and EphA4 null mutant (o/o) mice. (A). Schematic representation of the corticospinal projection traced in mice. Multiple injections of the tracer was made in the motor cortex in the left cerebral hemisphere of adult mice. The labelled CST axons descend through the midbrain, pons and pyramid in the medulla. In wild type and heterozygous mice, the CST axons decussate at the medulla, crossing the midline travelling from left ventral to right dorsal, enter the dorsal funiculus of the spinal cord and terminate predominantly in the dorsal horn contralateral to the tracer injections. In EphA4 null mutant mice, labelled CST axons appeared to terminate in the medulla and intermediate and ventral region of the spinal grey matter. Some labelled fibres were observed to recross the midline. PAG, periaqueductal grey; ICP, inferior cerebellar peduncle; V, trigeminal nucleus; IO, inferior olive; RN, red nucleus; NRM, nucleus raphe magnus; VII, facial nucleus; cun, cuneate nucleus. (B) Transverse sections of medulla showing the decussation of labelled CST fibres travelling from left ventral (v) to right dorsal (d). In EphA4 o/o mice, many CST axons do not enter the dorsal column area. Scale bar = 450 μ m. (C and D) Transverse sections of cervical spinal cord showing area of dorsal funiculus (C) and dorsal horn (D). In wild type animals, labelled CST axons terminate in the right dorsal horn (arrow). In homozygotes, axons project predominantly into the intermediate and ventral regions of the grey matter, and no labelled axons were observed terminating in the dorsal horn. cc, central canal; df, dorsal funiculus. Scale bar = 125 μ m. (E) Longitudinal sections of cervical spinal cord. CST axons

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in the right dorsal funiculus are seen in the midline. In homozygous animals some CST fibres recross the midline and project to the grey matter ipsilateral to the tracer injections. Scale bar = $300\mu\text{m}$. (F) Transverse sections of lumbar spinal cord. A reduced number of labelled CST axons was observed in the dorsal funiculus of the null mutant mice compared to normal. Scale
5 bar = $125\mu\text{m}$.

Figure 4 is a photographic representation showing analysis of EphA4 expression in wild type neonatal mouse tissues by immunohistochemistry (A) and in situ hybridization (B and C). (A) Coronal section of the medulla stained with anti-EphA4 antibody. EphA4 was detected in the
10 inferior olivary nucleus (ol), but not in the pyramidal tract (py). Scale bar = $125\mu\text{m}$. (B) Dark-field photomicrograph showing a coronal section of brain hybridized with radiolabelled-antisense EphA4 probe. The level of EphA4 mRNA within the sensorimotor cortex (sm) region is not above background. Scale bar = $420\mu\text{m}$. (C) Dark-field, and (D) bright-field, photomicrograph of cervical spinal cord transverse section hybridized with antisense EphA4
15 probe. EphA4 mRNA is found expressed within the intermediate and ventral regions of the spinal cord grey matter. df, dorsal funiculus. Scale bar = $150\mu\text{m}$. No signal was observed in equivalent tissue sections stained with radiolabelled-sense probe.

Figure 5 is a photographic representation showing analysis of EphrinB3 expression in wild
20 type E18.5 mouse tissue by in situ hybridization. Coronal sections of whole head were hybridized with DIG-labelled (A) antisense Ephrin B3 and (B) sense riboprobes. An intense signal of Ephrin B3 mRNA is detected within the sensorimotor (sm) cortex region. Scale bar = $400\mu\text{m}$.

25 **Figures 6A to C** are photographic representations defining the localisation of EphA4 expression in the brain and spinal cord of a 17.5 day old mouse wild type embryo.

Figures 7A and B are photographic representations defining the localisation of EphA4 protein in cross sections of the adult spinal cord after a trauma injury (A) and in an uninjured
30 control (B).

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EXAMPLE 1

MATERIALS AND METHODS

Targeted disruption of EphA4 gene. For homologous recombination, 5' *HindIII-SacI* 3kb sequence and 3' *Eco47III-BamHI* 5.5kb sequence flanking exon III were subcloned into the pKJ1 vector (Fig 1). The vector contains the neomycin-resistance gene (*neo*) with the phosphoglycerate kinase (*pgk*) promoter and *pgk* polyadenylation signal. The W9.5 embryonic stem cell line was electroporated with the *Sal* I linearized targeting construct and selected with G418 for 10 days. A total of 480 surviving clones were expanded and homologous recombinants were identified by Southern analysis of genomic DNA from single clones digested with *EcoR*I. Two isolated clones with a single targeted mutation of EphA4 gene were each injected into (C57BL/6 x C57BL/10) F_2 blastocysts. Chimeras were mated to C57BL/6 mice to produce heterozygotes. Southern analysis of tail DNA was used for genotyping the offspring.

15

Whole-mount and Tissue Immunocytochemistry and PCR Genotyping of Embryos.

Whole-mount immunocytochemistry was performed with anti-EphA4 antibody (available from D.G. Wilkinson of NIMR, Mill Hill, UK) as previously described (19) and colour detection was carried out using BCIP/NBT (Promega) as substrate. For tissue sections, tissues were fixed for 24 hours in 4% v/v paraformaldehyde and then another 24 hours in fixative containing 30% w/v sucrose. Frozen tissue was serially sectioned 50 μ m thick. Immunohistochemistry was performed using anti-EphA4 antibody and the same protocol as for whole mounts, except the ABC Elite detection system (Vector Laboratories, Burlingame CA) was used to detect colour staining.

25

Embryos were genotyped by PCR of yolk sac DNA (20) using primer pairs P1 CGTGCTACTTCCATTTGTCACGTCCTG [SEQ ID NO:1] and P2 TGCCGTGATAGCAAATTTGAG [SEQ ID NO:2] or P3 AGGAAGTGAGCATTATGGATGA [SEQ ID NO:3] and P4 TGCTCCTCGTGCCCAGCGTT [SEQ ID NO:4]. A 600bp band is generated from the mutant allele between the neomycin primer P1 and *ephA4* endogenous primer P2; a 645bp product is

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generated from the wild type allele between exon III primers, P3 and P4. The PCR reaction was in a total volume of 50 μ l and consisted of 50-500ng DNA, 30pmoles of each primer, 2.0mM MgCl₂, 100 μ M dNTPs, 1 U Taq polymerase (Roche) with the appropriate reaction buffer supplied by the manufacturer. The cycling reaction was 15 cycles of 96 $^{\circ}$ C for 30 sec, 5 70 $^{\circ}$ C for 30 sec (-1 $^{\circ}$ C per cycle) and 72 $^{\circ}$ C for 1 min, followed by 20 cycles of 96 $^{\circ}$ C for 30 sec, 55 $^{\circ}$ C for 30 sec, and 72 $^{\circ}$ C for 1 min.

Histology. Histological examination was carried out on EphA4 homozygous, heterozygous and wild type littermates of embryonic age E16, 8 day and 24 day old mice. Embryos and 10 adult tissues were fixed overnight in 10% v/v formalin, paraffin-embedded and serially sectioned 4 μ m thick. Sections were stained with either haematoxylin and eosin or luxol fast blue.

In-situ Hybridization. For EphA4 mRNA expression, tissues were fixed overnight in 10% 15 formalin, paraffin-embedded and serially sectioned 4 μ m thick. In situ hybridization was performed as previously described (21) using ³³P-radiolabeled complimentary EphA4 RNA probe. The antisense probe was synthesized with T7 polymerase from the *Hind*III-linearized plasmid Bluescript KS, containing a 1.5kb *Eco*RI fragment of 3' untranslated and C-terminal coding sequences of EphA4 (provided by D.G. Wilkinson of NIMR, Mill Hill, UK).

20

For expression of EphrinB3 mRNA, DIG-labelled in situ hybridization was performed on frozen 20 μ m tissue sections as previously described (22). To generate the Ephrin B3 probe, Ephrin B3 cDNA was amplified by PCR from adult mouse brain cDNA, using primers TTAGAATTCCCCGAGGAGGAGCTGTAC [SEQ ID NO:5] and 25 CTAGAATTCTGCAGTCCCACCAACCCCG [SEQ ID NO:6]. The PCR product, which spans 551bp to 953bp of Ephrin B3 cDNA (13), was cloned into *Eco*RI site of Bluescript SK and sequenced. The antisense probe was then synthesized with T3 polymerase from the *Hind*III-linearized plasmid.

30 **Surgery, Anterograde Tracing and Tissue Processing.** Corticospinal axons and their terminal projections were labelled in 5 week old mice using the anterograde tracer,

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Biotinylated Dextran Amine (BDA, 15%) (Molecular Probes, Eugene, ON). Two wild type, one heterozygous, and three homozygous EphA4 mutant mice were used for these studies. The animals were anaesthetised by injecting intraperitoneal (10 μ l/gm body weight) a 1:1:6 ratio mixture of Hypnorm (Janssen, Oxford, UK), Hypnovel (Roche), and distilled H₂O.

5 Anaesthetised animals had their head positioned in a stereotaxic frame and a craniotomy (3-4 mm in diameter) was made to expose the rostral half of the left cerebral hemisphere. Seven injections of 0.3 μ l of tracer were made into the cerebral cortex at a depth of 0.5 - 1.0 mm below its surface using a glass pipette (tip diameter 50 μ m) attached to a Hamilton syringe (23). The injections covered the whole sensorimotor region of the cerebral cortex. The

10 number of injections, the injection sites and the amount of tracer used per injection were kept consistent between control and mutant animals. The brain and spinal cord were perfused 7 days following the injection with 0.9% w/v phosphate buffered saline and 4% v/v paraformaldehyde in phosphate buffer (PB). The tissue was postfixed for 24 hours in 30% w/v sucrose in buffered fixative.

15

The free-floating sections were processed according to the method as described (24) in order to visualise the axons and terminals labelled by BDA. Phosphate buffer (0.1M) was the vehicle for the immunoreagents and for rinsing after each of the following steps: (a) incubation in 0.3% v/v hydrogen peroxide in methanol for 20 mins to block any endogenous peroxidase

20 activity (b) incubation in Avidin-peroxidase (Sigma) diluted 1:5,000 in 0.1M phosphate buffer and 0.75% v/v Triton X-100 for 2 hours (c) processing for horseradish peroxidase histochemistry using cobalt-enhanced diaminobenzidine (DAB) reaction (25) for 8-10 mins. This process stained the axons and terminals labelled with BDA black. Transverse spinal cord sections were counter-stained with haematoxylin.

25

EXAMPLE 2

GENERATION OF EPHA4 HOMOZYGOUS MICE

EphA4 deficient mice were generated using targeted mutagenesis and embryonic-stem (ES)

30 cell technology (26). The gene targeting strategy (Fig 1A) replaces exon III with a neomycin selection gene thereby introducing a frame shift and stop codon in the *ephA4* gene. To

- 20 -

demonstrate that the EphA4 mutation results in a null mutation, whole-mount immunohistochemistry was performed on E8.5 embryos (Fig 1C). In wild type and heterozygous embryos, EphA4 was expressed in rhombomeres 3 and 5 (arrows), as previously described (14). In contrast, no staining was observed in the EphA4 homozygous embryos. The
5 antibody recognises the C-terminus of the intracellular domain (2783-3195 residues) of EphA4 (19) and thus, the lack of staining observed in the homozygous embryos implies that no EphA4 protein is produced in these mutant mice. EphA4 null mutant mice generated from two independent ES cell lines were viable and fertile. The number of EphA4 homozygous mice in litters born from crossing heterozygotes showed a normal Mendelian ratio (25%), indicating
10 no lethality of the mutation during embryogenesis.

EXAMPLE 3

EPHA4 HOMOZYGOUS MICE DISPLAY AN ABNORMAL HOPPING GAIT

15 The EphA4 null mice exhibited locomotor abnormalities with impairment of the co-ordinated movement of the limbs. Both mouse strains showed hesitation in initiating locomotion, and once they began to move there was lack of the normal synchronous movement of each forelimb with the contralateral hindlimb. Most striking was an abnormal, synchronous, "kangaroo-like" movement of the hindlimbs while reciprocal movement of the forelimbs was maintained. In
20 contrast, the heterozygous mice showed no abnormality.

Tests of neurological function were performed to further characterize the defects in these animals. The hesitation to move and lack of co-ordination in the hindlimbs was reflected in open field activity tests (27) which showed the distance travelled by the EphA4 homozygotes
25 was only 30% of the heterozygote value (EphA4 homozygotes crossed 18 ± 24 grids per 5 minutes compared to heterozygous littermates which crossed 60 ± 34 grids, $n = 15$, $p < 0.0005$). In addition, the EphA4 null mutant animals showed placing deficits of both hindlimbs, suggesting a defect in corticospinal projections (28, 29), whereas sensory tests were within normal limits.

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EXAMPLE 4

DISRUPTION OF SPINAL CORD ARCHITECTURE AND THE ANTERIOR COMMISSURE IN EPHA4 HOMOZYGOUS MICE

5 Anatomical studies were performed to determine if there were major structural changes in the central nervous system of the EphA4 null mice. While there was no macroscopic abnormality, histological analysis of spinal cord sections showed that the dorsal funiculus was markedly shallower in the EphA4 null animals compared to heterozygous and wild type animals (Fig 2A). The major motor pathway, the corticospinal tract (CST), descends through the dorsal
10 funiculus in the rodent spinal cord. Anatomical studies revealed a further defect in the EphA4 null mutant mice, a loss of the anterior commissure (AC). This was observed in 12 of the 14 homozygous specimens examined (Fig 2B), but appeared normal in all heterozygous and wild type mice. No other anatomical abnormalities were observed in the brains of EphA4 mutants, including within the motor cortex, midbrain, and medullary pyramids.

15

EXAMPLE 5

CORTICOSPINAL PROJECTION IS ABERRANT IN EPHA4 HOMOZYGOUS MICE

20 Functional tests and the abnormality in the dorsal funiculus suggested that the CST may be disrupted or absent in EphA4 deficient mice. This possibility was explored using dye tracing studies. Corticospinal axons were anterogradely labelled from their origin, layer V neurons in the motor cortex, to their terminal projections. Normally CST axons descend through the internal capsule, basis pedunculi in the midbrain, pons and medullary pyramids (Fig 3). In the
25 medulla the CST fibres cross the midline (decussate), then descend in the dorsal funiculus of the spinal cord and terminate predominantly in the dorsal horn contralateral to the cells of origin.

Anterograde labelling of corticospinal neurons in EphA4 null mice showed normal projection
30 within the fore- and mid-brain. However, the CST pathway within the medulla and spinal cord was clearly abnormal. It was observed in the medulla that, while many of the CST axons

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crossed the midline, a considerable number of axons appeared to terminate inappropriately at this level, so that a reduced number of axons descended in the dorsal column of the spinal cord (Fig 3B). In addition, those axons which descended in the dorsal funiculus showed an aberrant pattern of termination within the grey matter of the spinal cord (Fig 3C and 3D), with terminal
5 branches observed predominantly in the intermediate zone and ventral horn and very few terminals in the dorsal horn. A number of axons also recrossed the midline and terminated in the grey matter ipsilateral to the cortical tracer injection (Fig 3E). In the lumbar cord, there was a significant reduction in the number of CST axons (Fig 3F), making it difficult to demonstrate whether their pattern of termination was also aberrant at this level.

10

A small proportion of CST axons do not decussate in the medulla, but continue to descend ipsilaterally into the spinal cord in the ventral funiculus (30). The ipsilateral CST found within the ventral funiculus does not appear to be notably different in homozygous, heterozygous and wild type animals.

15

EXAMPLE 6

EXPRESSION OF EPHA4 AND LIGAND DURING CST DEVELOPMENT

To determine whether EphA4 protein was expressed in the CST, immunohistochemical studies
20 were undertaken on neonatal mouse brain tissues, which is the period when the CST projects through the medulla and enters the spinal cord (31, 32). EphA4 protein was not detected within the medullary pyramid or any other part of the CST at this age, however, it is expressed in the olivary nucleus which is dorsal to the pyramidal tract (Fig 4A). In addition, *in situ* hybridization studies were undertaken to determine whether EphA4 mRNA was detected
25 within the motor cortex, which is where the cell bodies of the CST are localized. Consistent with the immunohistochemistry data, *in situ* hybridization analysis shows levels of EphA4 mRNA within the sensorimotor cortex which are not above background (Fig 4B). However, a gradient expression of EphA4 mRNA was found within the spinal cord with high levels of expression detected in the intermediate and ventral regions of the spinal cord grey matter and
30 low levels of expression in the dorsal horns (Fig. 4C). This data indicate that EphA4 is not expressed in the CST axons but is found expressed in surrounding structures.

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To determine whether a ligand for EphA4 may be expressed in the CST, the inventors analysed the expression of Ephrin B3 within E18.5 mouse brain tissue (Fig 5). Of the transmembrane ligands, Ephrin B3 binds to EphA4 with the highest affinity (12, 13). *In situ* hybridization with the DIG-labelled Ephrin B3 antisense probe detected strong expression within the sensorimotor cortex region (Fig. 5A) thereby suggesting that Ephrin B3 is expressed in the motor neurons of the CST during its development.

EXAMPLE 7

EphA4 DOES NOT PLAY A SIGNIFICANT ROLE IN THE EARLY EMBRYONIC DEVELOPMENT OF THE CST

In this experiment, corticofugal axons are labelled with Dil in coronal sections of W/W and O/O E14 brains. Corticofugal axons in the O/O exhibited no obvious growth abnormalities at this stage and the number of processes reaching the internal capsule (IC) is not reduced, indicating that disruption of EphA4 does not greatly affect initial process guidance or neuronal viability.

EXAMPLE 8

EphA4 DOES NOT SIGNIFICANTLY AFFECT LUMBAR SPINAL CORD MOTONEURON SURVIVAL

Lumbar spinal motoneurons were retrogradely labelled in the W/W and O/O using the fluorescent tracers Tetramethylrhodamine and Fast Blue. Motoneurons of the O/O exhibited no obvious morphological differences when compared to the W/W. Furthermore, when the numbers of retrogradely labelled motoneurons that innervate the sciatic nerve are stereologically counted. Results indicated that there is no significant difference between the numbers of motoneurons in the W/W and O/O indicating that EphA4 may not be required for survival of some populations of lumbar spinal motoneurons.

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EXAMPLE 9**EphA4 MAY BE REQUIRED FOR THE CORRECT TOPOGRAPHIC
POSITIONING OF SOME LUMBAR SPINAL CORD
MOTONEURON POPULATIONS**

5

Labelling discrete lumbar spinal motoneuron populations with various fluorescent tracers revealed that in the O/O, the population of motoneurons that innervate the tibialis anterior muscle appear to have migrated further caudally than those observed in the W/W. In contrast, the motoneuron populations innervating the gastrocnemius muscle and sciatic nerve in the O/O
10 appear to be topographically similar to the homologous populations labelled in the W/W.

EXAMPLE 10**EphA4 IS EXPRESSED IN SPECIFIC AREAS DURING BRAIN DEVELOPMENT**

15 Shown in Figures 6A-C are photographic representations defining the localisation of EphA4 expression in the brain and spinal cord of a 17.5 day old mouse wild type embryo. Left panel: localisation of EphA4 receptor protein by immunohistochemistry using an EphA4 specific antibody and fluorescence staining. Right panel: localisation of EphA4 messenger RNA (mRNA) by *in situ* hybridisation using a radiolabeled EphA4 specific antisense probe (positive
20 labelling appears as clusters of white dots).

A. Cross section through the brain in the region of the hippocampus (Hip). EphA4 expression is found in the hippocampus, sensorimotor cortex (Sm) and the caudate putamen (Cp).

25 B. Cross section through the medullary region of the brain. EphA4 protein expression was not detected within the medullary pyramid (Py) or any other part of the CST during any stage of development, but both EphA4 mRNA and protein was found to be strongly expression in the olivary region (OR), which is directly dorsal to the pyramidal tract.

30 C. Cross section through the cervical spinal cord. EphA4 protein and mRNA is found in the intermediate and ventral regions of the spinal cord grey matter but not in the dorsal horns.

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These data indicate that EphA4 is not expression on the CST axons but is expression in the surrounding environment through which they grow.

EXAMPLE 11

5 **EphA4 PROTEIN IS EXPRESSED FOLLOWING SPINAL CORD TRAUMA**

Shown in Figures 7A and B are photographic representations defining the localisation of EphA4 protein in cross sections of the adult spinal cord after a trauma injury (A) and in an uninjured control (B). Strong EphA4 expression is found in the injured spinal cord
10 predominantly in the white matter (WM). The white matter surrounds the dorsal and ventral horns (DH and VH) of the spinal cord and is the area where axon tracts ascend and descend. Note that expression is found in dorsal funiculus (arrowhead) which is where the CST descends the spinal cord. No EphA4 expression is found in the uninjured spinal cord.

15 These data indicate that large areas of EphA4 expression after injury may inhibit regeneration of new axons in the adult spinal cord.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood
20 that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS

1. A method of facilitating regeneration, growth and/or development of a central nervous system in an animal or bird said method comprising increasing elevating or otherwise enhancing the levels of a Eph receptor or its functional equivalent or a ligand thereof.
2. A method of regulating axon guidance in an animal or bird said method comprising increasing, elevating or otherwise enhancing the levels of an Eph receptor or its functional equivalent or a ligand thereof.
3. A method for facilitating the repair or replacement of axons or otherwise facilitating repair of nervous tissue in animal or bird, said method comprising increasing, elevating or otherwise enhancing the levels of an Eph receptors or its functional equivalent in a region surrounding the cortex and/or inhibiting, reducing or otherwise down-regulating expression of the Eph receptor or its functional equivalent when expressed in tissues outside said region surrounding the cortex and which expression leads to blockage of axonal growth.
4. A method according to any one of claims 1 to 3 wherein the animal is a mammal.
5. A method according to claim 4 wherein the mammal is a human.
6. A method according to any one of claims 1 or 3 in response to disease or trauma.
7. A method according to claim 6 wherein the disease or trauma is brain or spinal cord injury or disease of the upper motor neuron.
8. A method according to claim 1 or 2 wherein the Eph ligand is an ephrin or a functional equivalent thereof.

9. A method according to any one of claims 1 to 3 comprising administering soluble or near soluble forms of Eph receptors or their ligands or their functional equivalents or multimeric forms thereof.
10. A method according to claim 9 wherein Eph receptor or ligand administration is *via* the spinal cord or brain.
11. A method according to claim 10 wherein Eph receptor or ligand administration is facilitated by co-administration with the TAT gene of HIV.
12. A method according to claim 11 wherein Eph receptor or ligand administration is *via* intraperitoneal, intravenous or subcutaneous administration.
13. A method according to any one of claims 1 to 3 wherein the Eph receptor is EphA4.
14. A genetically modified animal producing reduced levels of an Eph receptor or ligand thereof or their functional equivalents.
15. A genetically modified animal producing elevated levels of an Eph receptor or ligand thereof or their functional equivalents.
16. A genetically modified animal according to claim 14 or 15 wherein the Eph receptor is EphA4 or its functional equivalent.
17. A genetically modified animal according to claim 14 or 15 or 16 wherein the animal is a murine species.
18. A composition useful in facilitating regeneration, growth and/or development of a control nervous system or regulating axon guidance or facilitating repair or replacement of axons or repair of nervous tissue said composition comprising an Eph receptor or ligand or

a functional equivalent thereof and one or more pharmaceutically acceptable carriers and/or diluents.

19. A composition according to claim 18 wherein the Eph receptor is EphA4.

20. A composition according to claim 18 wherein the ligand is an ephrin.

21. Use of an Eph receptor or its ligand or their functional equivalently in the manufacture of a medicament for the regeneration, growth and/or development of a central nervous system or for regulating axon guidance or for facilitating repair or replacement of axons or for facilitating repair of nervous tissue.

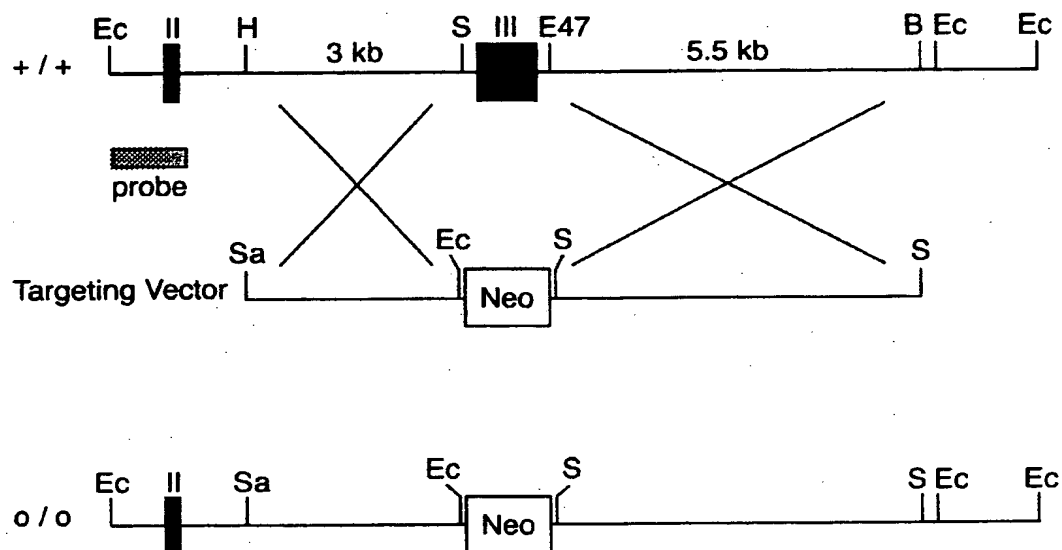
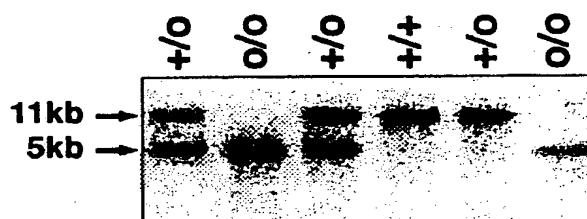
A**B****C**

Figure 1
Substitute Sheet (Rule 26) RO/AU